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Concise Review: Human Embryonic Stem Cells—What Have We Done? What Are We Doing? Where Are We Going?

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Key Words. Human embryonic stem cells • Induced pluripotent stem cells • Clinical trials • Pluripotent stem cells

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ABSTRACT

Human pluripotent stem cells possess remarkable proliferative and developmental capacity and thus have great potential for advancement of cellular therapy, disease modeling, and drug discovery. Twelve years have passed since the first reported isolation of human embryonic stem cell lines (hESC), followed in October 2010 by the first treatment of a patient with hESC-based cellular therapy at the Shepherd Center in Atlanta. Despite seemingly insurmountable challenges and obstacles in the early days, hESC clinical potential reached application in an extraordinarily short time. Eight currently ongoing clinical trials are yielding encouraging results, and these are likely to lead to new trials for other diseases. However, with the discovery of induced pluripotent stem cells (iPSC), disease-specific hESC lines derived from patients undergoing preimplantation genetic diagnosis for single gene disorders fell short of expectations. Lack of ethical controversy made human iPSC (hiPSC) with specific genotypes/phenotypes more appealing than hESC for drug discovery and toxicology-related studies, and in time, lines from HLA-homologous hiPSC banks are likely to take over from hESC in clinical applications. Currently, hESC are indispensable; the results of hESC-based clinical trials will set a gold standard for future iPSC-based cellular therapy. STEM CELLS *2017;35:17–25*

SIGNIFICANCE STATEMENT

hESC-based therapies have now become a reality. However, the development of HLA-homozygous iPSC banks, such as the one in Japan provide an ethically neutral alternative to hESC for therapeutic as well as research applications. International guidelines on screening and application of these iPSC lines will likely lead to complete redundancy of hESC lines at some point in the future.

INTRODUCTION

Optimism that human embryonic stem cells (hESC) would provide a virtually unlimited source of selected cell types for future cell therapies, as well as drug screening and development, has resulted in a considerable progress in stem cell biology over nearly two decades since the first hESC were derived [1]. However, the controversy over the use of hESC in research and translational medicine has not diminished over time. There is a constant clash between the obligation to protect life and the obligation to help and save those who are suffering. The very strong opinions on the moral standing of human embryos have led to the prohibition of work with hESC in some countries or, where allowed, this work is tightly regulated.

CIRCUMVENTING ETHICAL CONTROVERSY—HESC LINES FROM SINGLE BLASTOMERES

Ethical controversy determined the direction of early work; this focussed on how to establish hESC lines without destruction of the embryo. A team from Advanced Cell Technology, a Massachusetts-based company, succeeded in deriving hESC lines from single blastomeres of cleavage stage embryos [2]. In this proof-of-principle study the embryos did not survive. To minimize the number of embryos used, the embryos were disaggregated and all blastomeres were sourced for derivation. In the follow-up study [3], one or two blastomeres were biopsied from cleavage stage embryos, and the remaining embryo was left to develop to blastocyst stage. This strategy mimicked preimplantation genetic diagnosis (PGD), a routine assisted reproduction procedure for selection of

healthy embryos for transfer and elimination of the embryos carrying disease-linked mutations. In spite of addressing the major ethical concerns, the technique did not become widespread. In the same year that the detailed protocol was published [4], two groups generated the first human induced pluripotent stem cell (hiPSC) [5, 6], and all the excitement around hESC started to fade—hESC were seen almost as an historical anomaly.

Instead of being celebrated as a major achievement, the technique of hESC-derivation from single blastomeres without embryo destruction became a center of controversy per se. Following nearly 50,000 public comments on the published draft *Guidelines for research involving hESCs*, the NIH modified the definition of hESCs [7]. hESC "are cells that are derived from the inner cell mass of blastocyst stage human embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers." Under these guidelines, five hESC lines derived from single blastomeres by the Advanced Cell Technology team (MA09, NED1-4) [2, 3] and ten lines derived at the University of California San Francisco (UCSFB1-10) [8] were ineligible for review because they were derived from a preblastocyst stage embryo and therefore, according to the definition, are not considered to be hESC lines [9]. Applications were submitted to the NIH hESC Registry in 2009 and 6 years later the decision is still pending. Paradoxically, there were no questions raised when clinical trials for macular degeneration of retina using MA09-derived retinal pigment epithelial (RPE) cells were labeled as hESC-based cellular therapy [10].hESC generated without embryo destruction changed views on hESC patentability in the EU. Following the Directive 98/44/EC on the Legal Protection of Biotechnological Inventions [11] the European Patent Office (EPO) has refrained from granting patents for hESC on moral grounds. In 2014, the EPO's Technical Board of Appeal decided that Chung et al. [3] provided the first disclosure of a method of establishing hESC lines without destroying a human embryo on 7 February 2008. Since then, only the hESC-related applications filed before that date were excluded from patentability [12].

hESC Lines Carrying Disease-Specific Mutations

hESC derived from embryos carrying monogenic inherited diseases or chromosomal aberrations were seen as tools for elucidating the etiology and pathophysiology of disorders. On that premise more than 100 hESC lines have been derived. Most of them were listed on either NIH hESC Registry or Human Pluripotent Stem Cell Registry. The spectrum of diseases was limited by the availability of PGD treatments and the frequency of the specific mutations in a given population (Table 1). The most frequently derived were hESC lines carrying specific mutations linked to Huntington disease (21 lines derived in 8 centers), Fragile X syndrome (12 lines derived in 3 centers), cystic fibrosis (12 lines derived in 6 centers), myotonic distrophy (11 lines derived in 6 centers), and Charcot–Marie–Tooth disease (11 lines derived in 5 centers). In spite of efforts to make such lines available to the scientific community, actual interest did not match the initial enthusiasm. A relatively modest number of publications in peer-reviewed journals have described their use as research tools; in fact, the number of reviews elaborating on opportunities of using hESC lines carrying specific disease-linked mutations was several times higher than the number of actual research papers. Ethical issues, the regulatory landscape, and the limited spectrum of diseases were all drawbacks of hESC lines that hiPSC did not have and not surprisingly, disease-specific hiPSC lines took over.

Clinical Grade hESC Lines

Clinical grade hESC lines are lines which have been derived under current Good Manufacturing Practice (cGMP) conditions. The first clinical-grade hESC lines were the result of international efforts. Cryopreserved embryos were donated at Sydney IVF Ltd., derivation was performed at a cGMP facility in Brisbane, Australia, and the project was sponsored by the company ES Cell International, which was at that time based in Singapore [13]. The research versions of these lines were available for minimal reimbursement through the A*STAR Singapore Stem Cell Consortium (SSCC). Despite multimillion investments in these first clinical grade hESC lines, they did not gain the popularity of the H1 and H9 hESC lines derived by Thomson et al. [1], and the cells were never used in clinical trials. Since May 2010, the lines are owned by the California-based company BioTime. The company further characterized the lines at the molecular level and made the data, including copy number variation and genome sequencing, publicly available [14].

In the U.K., more than 30 clinical grade hESC lines have been derived in five centers across the country as a result of systematic investment from the Medical Research Council. The results of molecular karyotyping of 25 UK-derived clinical-grade hESC lines by whole-genome single nucleotide polymorphism array analysis was recently published [15]. Fifteen unique copy number variants greater than 100 kb and three copy-neutral regions of loss of heterozygosity greater than 1 Mb were detected in these 25 lines; none of these was associated with adaptation to cell culture. The presence of the culture artefact microduplication of chromosome 20q11.21 was, however, found at higher passages of four clinical grade hESC lines. The methodology and the results of testing cell lines for human viral pathogens has been made available for only 2 of these 25 lines, KCL033 and KCL034 [16].

Whether further investments into characterization of large numbers of hESC lines might pay off, only time will tell, especially with the expanding HLA-homozygous iPSC bank in Japan for clinical purposes [17]. The bank will contain multiple clinical grade iPSC lines homozygous for three HLA loci: HLA-A, -B, and -DR. Since autologous iPSC-based cell therapy would be financially prohibitive, the aim is to derive the lines from donors homozygous for HLA haplotypes that are found in the Japanese population at a high frequency. The cells derived from such hiPSC lines will carry a reduced risk of rejection when transplanted into recipients that are heterozygous for these haplotypes. Since Japan has a relatively homogenous ethnic population, the required size of the Japanese HLA-homozygous iPSC bank seems to be relatively small—about 50 homozygous lines will match >90% of the Japanese population [18, 19]. In the ethnically more diverse U.K., among 405 theoretical homozygous HLA combinations, a tissue bank

Table 1. PGD lines listed hPSC and NIH hESC Registry.

Disease	Line	Human pluripotent stem cell registry	NIH	Institution	
Adrenoleuko-dystrophy	SI-201	RGIe105-A		Reproductive Genetics Institute	USA
	UM112-1 PGD		NIHhESC-14-0285	University of Michigan	USA
	UM112-2 PGD		NIHhESC-15-0307		
Alpha thalassemia	GENEA073		NIHhESC-12-0193	Genea	Australia
Alport syndrome	Lis14_Alport_3		NIHhESC-15-0340	Tel Aviv Sourasky Medical Center	Israel
Amyotrophic lateral sclerosis; frontotemporal dementia	UM141-6 PGD		NIHhESC-16-0360	University of Michigan	USA
Androgen insensitivity	Lis07_AIS_1		NIHhESC-15-0334	Tel Aviv Sourasky Medical Center	Israel
	Lis08_AIS_2		NIHhESC-15-0335		
Aniridia (PAX6)	UM29-2 PGD		NIHhESC-12-0164	University of Michigan	USA
	UM29-3 PGD		NIHhESC-12-0165		
Becker muscular dystrophy	SI-170	RGIe077-A		Reproductive Genetics Institute	USA
Beta Thalassemia	SI-158	RGIe066-A		Reproductive Genetics Institute	USA
	SI-164	RGIe072-A			
	OZ-8	IMHe011-A		Istanbul Memorial Hospital	Turkey
	KCL035		NIHhESC-13-0227	King's College London	U.K.
Becker muscular dystrophy	GENEA058		NIHhESC-12-0199	Genea	Australia
BRCA1	GENEA059		NIHhESC-12-0175	Genea	Australia
Charcot-Marie-tooth disease type 1	VUB20_CMT1A	VUBe014-A		Vrije Universiteit Brussel	Belgium
	STR-I-315-CMT1a	INSRMe015-A		INSERM	France
	HUES PGD 11		NIHhESC-11-0094	Harvard University	USA
	HUES PGD 12		NIHhESC-11-0095		
	UM11-1PGD		NIHhESC-12-0153	University of Michigan	USA
	UM59-2 PGD		NIHhESC-14-0275		
	UM59-4 PGD		NIHhESC-16-0357		
	UM89-3 PGD		NIHhESC-16-0358		
	GENEA064		NIHhESC-12-0174	Genea	
	GENEA062		NIHhESC-12-0187		
	GENEA063		NIHhESC-12-0188		
Cystic fibrosis	KCL003	KCLe003-A		King's College London	U.K.
	KCL021		NIHhESC-13-0219		
	KL042		NIHhESC-13-0242		
	KL043		NIHhESC-13-0243		
	STR-I-203-CFTR	INSRMe008-A		INSERM	France
	STR-I-251-CFTR	INSRMe009-A			
	SI-257	RGIe156-A		Reproductive Genetics Institute	USA
	VUB04_CF	VUBe004-A		Vrije Universiteit Brussel	Belgium
	VUB22_CF	VUBe015-A			
	HAD 2	HADe002-A		Hadassah University Hospital	Israel
	GENEA041		NIHhESC-12-0167	Genea	Australia
	GENEA040		NIHhESC-12-0171		
Duchenne muscular dystrophy	SI-180	RGIe086-A		Reproductive Genetics Institute	USA
	HUES PGD 3		NIHhESC-11-0091	Harvard University	USA
	Lis48_DMD_6_N		NIHhESC-15-0311	Tel Aviv Sourasky Medical Center	Israel
	Lis23_DMD_5		NIHhESC-15-0328		
	Lis10_DMD_1		NIHhESC-15-0337		
	Lis11_DMD_2		NIHhESC-15-0338		
	Lis20_DMD_3		NIHhESC-15-0345		
	Lis22_DMD_3		NIHhESC-15-0347		
Emery-Dreifuss muscular dystrophy	SI-245	RGIe144-A		Reproductive Genetics Institute	USA
Fabry disease	STR-I-171-GLA	INSRMe004-A		INSERM	France
Facioscapulohumeral muscular dystrophy	VUB09_FSHD	VUBe009-A		Vrije Universiteit Brussel	Belgium
	GENEA024		NIHhESC-12-0170	Genea	Australia
	GENEA049		NIHhESC-12-0183		

Table 1. *Continued*

Disease	Line	Human pluripotent stem cell registry	NIH	Institution	
	GENEA050		NIHhESC-12-0184		
	GENEA096		NIHhESC-14-0244		
Factor VIII deficiency	HAD 3	HADe003-A		Hadassah University Hospital	Israel
Familial adenomatous polyposis	STR-I-305-APC	INSRMe014-A		INSERM	France
	STR-I-355-APC	INSRMe017-A			
	STR-I-359-APC	INSRMe018-A			
	Lis34_FAP_3		NIHhESC-15-0324	Tel Aviv Sourasky Medical Center	Israel
	Lis34_FAP_2		NIHhESC-15-0325		
	Lis25_FAP_1		NIHhESC-15-0349		
Fanconi's anemia	SI-128	RGIe040-A		Reproductive Genetics Institute	USA
Fragile X syndrome	WCMC-37	NIHhESC-13-0211		Weill Cornell Medical College	USA
	UM139-2 PGD		NIHhESC-14-0292	University of Michigan	USA
	Lis 51_FXS9_N		NIHhESC-15-0309	Tel Aviv Sourasky Medical Center	Israel
	Lis39_FXS8_N		NIHhESC-15-0319		
	Lis38_FXS7_N		NIHhESC-15-0320		
	Lis37_FXS10_N		NIHhESC-15-0321		
	Lis29_FXS_7		NIHhESC-15-0326		
	Lis01_HEFX1		NIHhESC-15-0329		
	Lis02_FXS_2		NIHhESC-15-0330		
	Lis03_FXS_4		NIHhESC-15-0331		
	Lis24_FXS_5		NIHhESC-15-0348		
	Lis26_FXS_6		NIHhESC-15-0350		
Hemophilia B	UM9-1PGD		NIHhESC-12-0154	University of Michigan	USA
Hereditary multiple exostoses	ES-11EM	ESe026-A		Spanish Stem Cell Bank	Spain
	GENEA097		NIHhESC-14-0248	Genea	Australia
	GENEA098		NIHhESC-14-0249		
Huntington's disease	SI-186	RGIe091-A		Reproductive Genetics Institute	USA
	SI-187	RGIe092-A			
	SI-194	RGIe098-A			
	VUB05_HD	VUBe005-A		Vrije Universiteit Brussel	Belgium
	VUB28_HD_MFS	VUBe018-A			
	STR-I-155-HD	INSRMe003-A		INSERM	France
	KCL005	KCLe004-A		King's College London	U.K.
	KCL012	KCLe009-A	NIHhESC-13-0213		
	KCL013	KCLe010-A	NIHhESC-13-0214		
	KCL027		NIHhESC-13-0223		
	KCL036		NIHhESC-13-0241		
	KCL028		NIHhESC-13-0224		
	HUES PGD 16		NIHhESC-12-0150	Harvard University	USA
	UM17-1 PGD		NIHhESC-12-0160	University of Michigan	USA
	GENEA017		NIHhESC-12-0166	Genea	Australia
	GENEA018		NIHhESC-12-0169		
	GENEA046		NIHhESC-12-0180		
	GENEA090		HhESC-14-0245		
	GENEA091		HhESC-14-0246		
	GENEA089		HhESC-14-0247		
	HS799		NIHhESC-13-0207		Sweden
Hydrocephaly	Lis50_Hydrocephaly_3_N		NIHhESC-15-0308	Tel Aviv Sourasky Medical Center	Israel
	Lis49_Hydrocephaly_2_N		NIHhESC-15-0310		
	Lis35_Hydrocephaly_1		NIHhESC-15-0323		
Hydroxysteroid dehydrogenase 4 deficiency	UM15-4 PGD		NIHhESC-12-0161	University of Michigan	USA
Hypertrophic cardiomyopathy (MYBPC3)	UM38-2 PGD		NIHhESC-12-0155	University of Michigan	USA
Hypochondroplasia	GENEA077		NIHhESC-12-0261	Genea	Australia
Incontinentia pigmenti	GENEA071		NIHhESC-12-0191	Genea	Australia
Infantile neuroaxonal dystrophy	GENEA065		NIHhESC-12-0200	Genea	Australia

Table 1. *Continued*

Disease	Line	Human pluripotent stem cell registry	NIH	Institution	
Ichthyosis	Lis		NIHhESC-	Tel Aviv	Israel
	46_Ichthyosis_2_N		15-0313	Sourasky Medical Center	
	Lis 45_Ichthyosis_1_N		NIHhESC-15-0314		
Juvenile retinoschisis	GENEA072		NIHhESC-12-0192	Genea	Australia
Klinefelter's syndrome	Royan H4	Rie004-A		Royan Institute	Iran
	KCL008	KCLe007-A		King's College London	U.K.
	FC018	CEBe001-A		Cellartis	Sweden
	BG01V	VIACe001-A-2		Viacyte (Novocell)	USA
	WA16	WISCe002-A	NIHhESC-11-0097	University of Wisconsin	USA
Leuko-encephalopathy	Lis 41_LTBL_N		NIHhESC-15-0317	Tel Aviv Sourasky Medical Center	Israel
Loeys-Dietz syndrome 2	GENEA083		NIHhESC-14-0256	Genea	Australia
	GENEA084		NIHhESC-14-0257		
Marfan syndrome	SI-154	RGIe062-A		Reproductive Genetics Institute	USA
	STR-I-301-MFS	INSRMe013-A		INSERM	France
	VUB08_MFS	VUBe008-A		Vrije Universiteit Brussel	Belgium
	MFS5		NIHhESC-10-0052	Stanford University	USA
	UM89-1 PGD		NIHhESC-14-0276	University of Michigan	USA
	UM89-4 PGD		NIHhESC-16-0359		
Merosin deficiency 1A	GENEA081		NIHhESC-14-0255	Genea	Australia
Multiple endocrine neoplasia-type 2A	STR-I-209-MEN2a	INSRMe006-A		INSERM	France
	STR-I-211-MEN2a	INSRMe007-A			
	UM57-1 PGD		NIHhESC-13-0208	University of Michigan	USA
Myotonic dystrophy	VUB03_DM1	VUBe003-A		Vrije Universiteit Brussel	Belgium
	VUB19_DM1	VUBe013-A			
	VUB24_DM1	VUBe017-A			
	HAD 1	HADe001-A		Hadassah University Hospital	Israel
	SI-148	RGIe057-A		Reproductive Genetics Institute	USA
	SI-153	RGIe061-A			
	KCL018	KCLe014-A	NIHhESC-12-0218	King's College London	U.K.
	GENEA066		NIHhESC-12-0189	Genea	Australia
	GENEA067		NIHhESC-12-0190		
	Lis12_DM_1		NIHhESC-15-0339	Tel Aviv Sourasky Medical Center	Israel
	Lis19_DM_2		NIHhESC-15-0344		
Nemaline myopathy 2	GENEA078		NIHhESC-14-0252	Genea	Australia
	GENEA079		NIHhESC-14-0253		
	GENEA080		NIHhESC-14-0254		
Neurofibromatosis	SI-137	RGIe049-A		Reproductive Genetics Institute	USA
	SI-138	RGIe050-A			
	SI-139	RGIe051-A			
	SI-140	RGIe052-A			
	SI-235	RGIe134-A			
	KCL024		NIHhESC-12-0220	King's College London	U.K.
	KCL025		NIHhESC-12-0221		
	Lis 47_NF1_2_N		NIHhESC-15-0312	Tel Aviv Sourasky Medical Center	Israel
	Lis 42_NF1_1_N		NIHhESC-15-0316		
Nonsyndromic deafness	Lis43_connexin_3_N		NIHhESC-15-0315	Tel Aviv Sourasky Medical Center	Israel
	Lis17_Connexin_1		NIHhESC-15-0342		
	Lis18_Connexin_2		NIHhESC-15-0343		
Noonan syndrome	Lis21_Noonan_1		NIHhESC-15-0346	Tel Aviv Sourasky Medical Center	Israel
Osteogenesis imperfecta	VUB23_OI	VUBe016-A		Vrije Universiteit Brussel	Belgium
Patau syndrome (trisomy 13)	SA002	CEBe034-A		Cellartis	Sweden
	Miz-hES13	MIZMe015-A		MizMedi Hospital (MIZM)	South Korea
	FY-hES-5	GZHMCe001-A		The Third Affiliated Hospital of Guangzhou Medical College	People's Republic of China
	SA002		NIHhESC-10-0086	Cellartis	Sweden

Table 1. *Continued*

Disease	Line	Human pluripotent stem cell registry	NIH	Institution	
	UCLA7		NIHhESC-12-0143		USA
Retinitis pigmentosa	GENEA085		NIHhESC-14-0250	Genea	Australia
Saethre-Chotzen syndrome	Lis04_Twist		NIHhESC-15-0332	Tel Aviv Sourasky Medical Center	Israel
Simpson Golabi Behmel syndrome	GENEA088		NIHhESC-14-0260	Genea	Australia
Spinocerebellar ataxia	VUB10_SCA7	VUBe010-A		Vrije Universiteit Brussel (VUB)	Belgium
	STR-I-221-Sca2	INSRMe010-A		INSERM	France
	UM134-1 PGD		NIHhESC-14-0286	University of Michigan	USA
Spinal muscular atrophy	HUES PGD 1		NIHhESC-12-0148	Harvard University	USA
	HUES PGD 13		NIHhESC-11-0090		
	HUES PGD 14		NIHhESC-11-0136		
	KCL026		NIHhESC-13-0222	King's College London	U.K.
Torsion dystonia	GENEA074		NIHhESC-12-0194	Genea	Australia
	Lis09_DYS_1		NIHhESC-15-0336	Tel Aviv Sourasky Medical Center	Israel
Tuberous sclerosis 2	GENEA086		NIHhESC-14-0258	Genea	Australia
	GENEA087		NIHhESC-14-0259		
Turner syndrome	KCL041		NIHhESC-14-0273	King's College London	U.K.
Vitelliform macular dystrophy	GENEA069		NIHhESC-12-0181	Genea	Australia
	GENEA070		NIHhESC-12-0182		
Von Hippel-Lindau disease/ syndrome	KCL015	KCLe011-A	NIHhESC-13-0215	King's College London	U.K.
	KCL016	KCLe012-A	NIHhESC-13-0216		
	KCL017	KCLe013-A	NIHhESC-13-0217		
	GENEA060		NIHhESC-12-0172	Genea	Australia
	GENEA061		NIHhESC-12-0173	Genea	Australia
Wiskott-Aldrich syndrome	HUES PGD 2		NIHhESC-12-0195	Harvard University	USA
	KCL029		NIHhESC-13-0225	King's College London	U.K.
X-kinked myopathy with excessive autophagy	STR-I-229-MTMX	INSRMe011-A			
	STR-I-231-MTMX	INSRMe012-A			
Wilm's tumor	GENEA068		NIHhESC-12-0168	Genea	Australia

from 150 selected homozygous HLA-typed volunteers could match 93% of the population [20]. However, among 10,000 HLA typed organ donors used in the study as a representative of the UK population, only 2% were identified as non-White ethnicity, whereas according to the 2011 census 12.8% of the population was non-White, which indicates the particular challenges in identifying suitable donors for the members of these communities [21].hESC lines, such as H1, MA09, and I6 on which are based most clinical trials today, were not clinical grade lines from the start. They were derived as research grade lines and, only later were adapted to cGMP conditions. Moreover, they were derived and propagated in the presence of mouse feeder cells and/or bovine serum. Xeno-free technology was developed later [22–28] and in 2011, a team from King's College London derived the first eight animal product-free clinical grade lines [26–28]. The lines are karyotyped at the molecular level [15]; they are also listed on the NIH hESC Registry and, therefore, eligible for use in NIH-supported research. The most recent advance is the use of a cell culture matrix containing a mixture of human recombinant laminin (LN)−521 and E-cadherin [29] to derive hESC lines from the inner cell mass of blastocysts and from single blastomere cells from cleavage stage embryos without a need to destroy the embryo. The LN-521/E-cadherin matrix allows clonal derivation, survival and long-term self-renewal of hESC under chemically defined animal product-free conditions without addition of ROCK inhibitors.

All hESC lines do not have equal developmental potential and that cannot be explained by epigenetic memory as with hiPSC lines. Some of the hESC lines have propensity toward mesodermal lineages, whereas other toward endoderm [30]. Thus, a screening of the multiple hESC lines for their differentiation propensity has become a standard approach in selection of lines for particular clinical trials. The yield of differentiated cells basically depends on propensity of the source and the efficacy of differentiation protocol. However, regardless of differentiation efficacy, unlimited supplies of hESC or hiPSC would finally give more desired cell types than any other source, and therefore make the most of invested capital.

Clinical Trials

Spinal Cord Injury

In spite of all the obstacles, which include a 21,000-page Investigational New Drug (IND) application with the FDA, the first patient was treated with an hESC-based cellular therapy product, oligodendrocyte progenitor cells 1 (OPC1), in a clinical trial at the Shepherd Center in Atlanta in October 2010,

only 12 years after hESC were isolated for the first time [1]. The clinical trial for spinal cord injury, sponsored by Geron, a California-based company, treated only five patients. The treatment did not cause serious adverse events, although motor or sensory neurological changes were not observed. The lack of obvious improvement in physical condition clashed with the high expectations of the public and the company's stock dropped nearly 60% in the nine months, from January to September 2011. In order to stay in business, lack of investment and support forced the company to end the trial prematurely and to close their stem cell program [31].

All Geron's assets were transferred to another Bay Area company, BioTime and its subsidiary Asterias Biotherapeutics, in 2013. Supported with a strategic partnership award from the California Institute for Regenerative Medicine and equity funding, Asterias reinitiated the clinical trial, and the first patient was treated in Atlanta in June 2015. The study is conducted at a total of up to eight centers in the United States. The AST-OPC1cells will be tested with three sequential escalating doses, the highest being 20×10^6 cells, in 13 patients with subacute, C-5 to C-7, neurologically complete cervical spinal cord injury. In February 2014, Asterias received Orphan Drug Designation from the U.S. Food and Drug Administration (FDA) for AST-OPC1, for the treatment of acute spinal cord injury. Orphan Drug Designation is granted to products that treat diseases affecting fewer than 200,000 people in the U.S., and it may provide the sponsor certain benefits and incentives, including a period of marketing exclusivity of 7 years from the first marketing application, if regulatory approval is received for the designated indication [32].

Macular Degeneration of the Retina

Macular degeneration of the retina is likely to be the first disease that could be, to some extent, successfully treated with hESC-based therapy. Easy accessibility with minimally invasive procedures, the subretinal space being immunoprivileged, and the fact that the stem cell transplant can be monitored regularly with noninvasive methods for structural engraftment (spectral-domain optical coherence tomography) and functional outcome (autofluorescence and visual acuity), make the eye an ideal target choice for initial hESC/hiPSC-based cellular therapies. Indeed, there are currently nine clinical trials with hESC and one with hiPSC-derived RPE cells [10, 31, 33–35]. The initial results and follow-up with a median time of 22 months are promising; however, we do not know how long the effects will last. Over time the hESC-derived RPE cells might succumb to the pathologically altered environment of a diseased eye and ameliorate the condition only temporarily. Nevertheless, using ocular indications as a target was an ingenious idea and it revived the field after Geron was forced to end the trial for spinal cord injury.

Diabetes

Clinical trials with hESC/iPSC-based therapy in type I diabetes have been anticipated for a long time. California company Viacyte (formerly known as Novocell) has spent a number of years developing their glucose-responsive insulin producing PEC-01 cells as well as Encaptra, an encapsulating drug delivery system made from porous cell-impermeable membrane. They are currently tested together as VC-01, islet replacement product candidate. VC-01 is the first stem cell-based treatment for type 1 diabetes to enter clinical testing and the first patient was treated in October 2014 at the University of California San Diego [31, 36, 37].

Heart Repair

A clinical study of a fibrin patch embedded with hESC-derived cardiac-committed CD15+ ISL-1+ progenitors transplanted into epicardium of the infarcted area and covered with an autologous pericardial flap commenced in autumn 2014 in France [31, 38]. Following the treatment, the first patient suffering from severe heart failure New York Heart Association (NYHA) functional Class III improved to NYHA Class I and remained stable NYHA Class I 6-months after the intervention [38]. This is the first hESC-based clinical trial that originated outside of the US and that is not driven by a for-profit company.

hESC-Derived Cancer Vaccine

In 2011, Geron reported the development and modification of hESC-derived dendritic cells with mRNA as a potential strategy for the induction of T-cell-mediated immunity [39, 40]. With discontinuation of the stem cell program, the assets related to antigen-presenting dendritic cells GRN-VAC1 and GRN-VAC2 were transferred to Asterias. GRN-VAC2, renamed AST-VAC2, are mature hESC-derived dendritic cells that express a modified form of telomerase, which permits enhanced stimulation of immune response. In September 2014, Asterias teamed up with the UK charity Cancer Research UK and its development and commercialization arm Cancer Research Technology to bring AST-VAC2 into clinical trials in patients with non-small cell lung cancer and in January 2016 has completed the transfer of its manufacturing processes to Cancer Research UK who will produce AST-VAC2 under cGMP conditions at their Biotherapeutics Development Unit.

The Future—Where We Are Going with hESC?

With the development of hiPSC, free of ethical issues [5, 6], hESC started to lose their unique appeal. Within a few years, from being an indispensable research tool, hESC dropped to the level of "gold standard" demonstrating that iPSC are equally useful for addressing certain research questions [e.g., [41–44]]. Only time will show whether they will remain as a "gold standard" or they will slowly become obsolete. Most of the issues that are relevant for hESC-based therapy also apply to iPSC [45]. Therefore, it is logical that the standards set in hESC-based clinical trials should be applicable to hiPSC-based clinical trials (e.g., clinical trials in macular degeneration of retina). Since the key difference between hESC and hiPSC is the potentially modified genomic and epigenetic state of hiPSC, additional standards such as DNA methylation analysis and medium-resolution array-comparative genomic hybridization should be applied in hiPSC-based trials.

Nonuniform epigenome transformation during reprograming is not the only issue that may affect the quality of hiPSC [46]. hiPSC are derived from adult somatic cells, which accumulate mutations over the lifespan of the donor [47].

Specific genetic and epigenetic footprints influence the molecular and functional properties of each hiPSC clone and might lead to misinterpretation of the results in, for example, drug screening studies. This is particularly important in studies with disease-specific iPSC lines. With newly discovered relatively precise genome editing techniques such as clustered regularly-interspaced short palindromic repeats (CRISPR)/Cas9 [48, 49], it is possible to repair mutations in disease-specific hiPSC lines and in such a way generate much better controls than native non-manipulated hESC. On the other hand, using CRISPR/Cas9, disease-specific mutations can be introduced in normal healthy hESC lines, avoiding a baggage of accumulated lifetime mutations, which are typical for disease-specific hiPSC, as well as preserving the native DNA methylation footprint of hESC, which is almost never completely matched in hiPSC.

Author Contributions

D.I. and C.O.: wrote the manuscript.

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References

1 Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–1147.

2 Klimanskaya I, Chung Y, Becker S et al. Human embryonic stem cell lines derived from single blastomeres. Nature 2006;444:481–485.

3 Chung Y, Klimanskaya I, Becker S et al. Human embryonic stem cell lines generated without embryo destruction. Cell Stem Cell 2008;2:113–117.

4 Klimanskaya I, Chung Y, Becker S et al. Derivation of human embryonic stem cells from single blastomeres. Nat Protoc 2007;2:1963–1972.

5 Takahashi K, Tanabe K, Ohnuki M et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–872.

6 Yu J, Vodyanik MA, Smuga-Otto K et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318:1917–1920.

7 Available at http://stemcells.nih.gov/policy/pages/2009guidelines.aspx. Accessed March 13, 2016.

8 Zdravkovic T, Nazor KL, Larocque N et al. Human stem cells from single blastomeres reveal pathways of embryonic or trophoblast fate specification. Development 2015;142:4010–4025.

9 Available at http://grants.nih.gov/stem_cells/registry/pending.htm. Accessed March 13, 2016.

10 Schwartz SD, Regillo CD, Lam BL et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: Follow-up of two open-label phase 1/2 studies. Lancet 2015;385:509–516.

11 Directive 98/44/EC of the European parliament and of the council of 6 July 1998 on the legal protection of biotechnological inventions. Official J 1998;L213:13–21. http://eur-lex.europa.eu/eli/dir/1998/44/oj

12 Kim YT, Shepherd M, Zvesper T. Patentability of human embryonic stem cells. Available at http://www.marks-clerk.com/Home/Knowledge-News/Articles/Human-embryonic-stem-cells-at-the-EPO.aspx#.Vu0BDsec_dQ. Accessed March 19, 2016.

13 Crook JM, Peura TT, Kravets L et al. The generation of six clinical-grade human embryonic stem cell lines. Cell StemCell 2007;1:490–494.

14 Funk WD, Labat I, Sampathkumar J et al. Evaluating the genomic and sequence integrity of human ES cell lines: Comparison to normal genomes. Stem Cell Res (Amst) 2012;8:154–164.

15 Canham MA, Van Deusen A, Brison DR et al. The molecular karyotype of 25 clinical-grade human embryonic stem cell Lines. Sci Rep 2015;5:17258.

16 Devito L, Petrova A, Miere C et al. Cost-effective master cell bank validation of multiple clinical-grade human pluripotent stem cell lines from a single donor. Stem Cells Transl Med 2014;3:1116–1124.

17 Saito MK, Matsunaga A, Takasu N et al. Donor recruitment and eligibility criteria for HLA-homozygous iPS cell bank in Japan. In: Ilic D, ed. Stem Cell Banking. New York: Springer, 2014:67–76.

18 Nakatsuji N, Nakajima F, Tokunaga K. HLA-haplotype banking and iPS cells. Nat Biotechnol 2008;26:739–740.

19 Nakajima F, Tokunaga K, Nakatsuji N. Human leukocyte antigen matching estimations in a hypothetical bank of human embryonic stem cell lines in the Japanese population for use in cell transplantation therapy. Stem Cells 2007;25:983–985.

20 Taylor CJ, Peacock S, Chaudhry AN et al. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. Cell Stem Cell 2012;11:147–152.

21 Office for National Statistics. 2011 Census: Ethnic group, local authorities in the United Kingdom. Available at http://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/bulletins/keystatisticsandquickstatisticsforlocalauthoritiesintheunitedkingdom/2013-10-11. Accessed: May 09, 2016.

22 Ellerström C, Strehl R, Moya K et al. Derivation of a xeno-free human embryonic stem cell line. Stem Cells 2006;24:2170–2176.

23 Rajala K, Lindroos B, Hussein SM et al. A defined and xeno-free culture method enabling the establishment of clinical-grade human embryonic, induced pluripotent and adipose stem cells. PLoS One 2010;5:e10246.

24 Galán A, Simón C. Human embryonic stem cells derived in xeno-free conditions. Methods Mol Biol 2012;873:13–32.

25 Tannenbaum SE, Turetsky TT, Singer O et al. Derivation of xeno-free and GMP-grade human embryonic stem cells—Platforms for future clinical applications. PLoS One 2012;7:e35325.

26 Ilic D, Stephenson E, Wood V et al. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy 2012;14:122–128.

27 Stephenson E, Jacquet L, Miere C et al. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. Nat Protoc 2012;7:1366–1381.

28 Jacquet L, Stephenson E, Collins R et al. Strategy for the creation of clinical grade hESC line banks that HLA-match a target population. EMBO Mol Med 2013;5:10–17.

29 Rodin S, Antonsson L, Niaudet C et al. Clonal culturing of human embryonic stem cells on laminin-521/E-cadherin matrix in defined and xeno-free environment. Nat Commun 2014;5:3195.

30 Osafune K, Caron L, Borowiak M et al. Marked differences in differentiation propensity among human embryonic stem cell lines. Nat Biotechnol 2008;26:313–315.

31 Ilic D, Devito L, Miere C et al. Human embryonic and induced pluripotent stem cells in clinical trials. Br Med Bull 2015;116:19–27.

32 Available at http://www.fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/HowtoapplyforOrphanProductDesignation/default.htm. Accessed March 31, 2016.

33 Schwartz SD, Hubschman JP, Heilwell G et al. Embryonic stem cell trials for macular degeneration: A preliminary report. Lancet 2012;379:713–720.

34 Song WK, Park KM, Kim HJ et al. Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: Preliminary results in Asian patients. Stem Cell Reports 2015;4:860–872.

35 Kimbrel EA, Lanza R. Current status of pluripotent stem cells: Moving the first therapies to the clinic. Nat Rev Drug Discov 2015;14:681–692.

36 Schulz TC. Concise review: Manufacturing of pancreatic endoderm cells for clinical trials in type 1 Diabetes. Stem Cells Transl Med 2015;4:927–931.

37 Agulnick AD, Ambruzs DM, Moorman MA et al. Insulin-producing endocrine cells differentiated in vitro from human embryonic stem cells function in macroencapsulation devices in Vivo. Stem Cells Transl Med 2015;4:1214–1222.

38 Menasché P, Vanneaux V, Hagège A et al. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: First clinical case report. Eur Heart J 2015;36:2011–2017.
39 Tseng SY, Nishimoto KP, Silk KM et al. Generation of immunogenic dendritic cells from human embryonic stem cells without serum and feeder cells. Regen Med 2009;4:513–526.
40 Nishimoto KP, Tseng SY, Lebkowski JS et al. Modification of human embryonic stem cell-derived dendritic cells with mRNA for efficient antigen presentation and enhanced potency. Regen Med 2011;6:303–318.
41 Petrova A, Celli A, Jacquet L et al. 3D in vitro model of a functional epidermal permeability barrier from human embryonic stem cells and induced pluripotent stem cells. Stem Cell Rep 2014;2:675–689.
42 Sundberg M, Bogetofte H, Lawson T et al. Improved cell therapy protocols for Parkinson's disease based on differentiation efficiency and safety of hESC-, hiPSC-, and non-human primate iPSC-derived dopaminergic neurons. Stem Cells 2013;31:1548–1562.
43 Mica Y, Lee G, Chambers SM et al. Modeling neural crest induction, melanocyte specification, and disease-related pigmentation defects in hESCs and patient-specific iPSCs. Cell Rep 2013;3:1140–1152.
44 Otsuji TG, Kurose Y, Suemori H et al. Dynamic link between histone H3 acetylation and an increase in the functional characteristics of human ESC/iPSC-derived cardiomyocytes. PLoS One 2012;7:e45010.
45 Ilic D, Stephenson E. Promises and challenges of the first clinical-grade induced pluripotent stem cell bank. Regen Med 2013;8:101–102.
46 Ohi Y, Qin H, Hong C et al. Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. Nat Cell Biol 2011;13:541–549.
47 Young MA, Larson DE, Sun CW et al. Background mutations in parental cells account for most of the genetic heterogeneity of induced pluripotent stem cells. Cell Stem Cell 2012;10:570–582.
48 Jinek M, Jiang F, Taylor DW et al. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. Science 2014;343:1247997.
49 Cong L, Ran FA, Cox D et al. Multiplex genome engineering using CRISPR/cas systems. Science 2013;339:819–823.